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RESEARCH ARTICLE

Testing of Some Canine Blood Types in Transfusion Compatibility Assessment

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ABSTRACT

Blood types were determined using SHIGETA (n=136) and DEA1.1 (n=25) kits, in two groups of dogs, consisting of patients that underwent blood transfusions and healthy donors. The tests were conducted in accordance with the procedures established by the manufacturers, using specific monoclonal antibodies kits, heparinized blood for the tube agglutination (TUBE) and slide (SLIDE) methods, and EDTA treated blood for the CARD and chromatographic (CHROM) methods. The clear expression of tube agglutination reaction in the SHIGETA kit provided a good detection of antigens. Positive reactions with anti-DEA1.1 were clear and evident with the CHROM test. SHIGETA tests revealed a predominance 1.1B (47.05%) of blood type, common in Rotweilers (81.81%) and Romanian Shepherds (73.68%) and group 1(-)B (24.26%), frequently found in German Shepherds (54.16%), these also representing an important source of compatible blood. DEA1.1 type test, revealed a high frequency of positive dogs (75%), associated with lower number of potential donors. Extrapolation of SHIGETA groups into the DEA system, confirmed the 1(-)B positive dogs as DEA 1.1 negative, and their prevalence in German Shepherds also confirmed their known tendency to be "ideal donors". The CHROME test showed a good efficiency in auto agglutination control and detecting DEA1.1 positive dogs, including patients with severe forms of anemia.

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INTRODUCTION

The canine blood types studies targeted the DEA (Dog Erythrocyte Antigen) system and led to the identification of more than 13 different types, 8 of them are well known and have available serums for typing. An important contribution to the development and the broadening of knowledge regarding canine blood typing and testing transfusion compatibility was made by SHIGETA researchers, which described a new blood antigenic system, composed of 9 different types (Giger *et al.*, 2005; Ognean *et al.*, 2005). In order to reduce the risk of fatal transfusion reactions, higher in cats than in dogs detection of incompatibility is required in both species, through blood type testing or crossmatching (Lanevschi and Wardrop, 2001). Also, implementing alternatives to allogenic blood transfusion is recommended (Prittie, 2010).

The purpose of the present study was to analyze the incidence of different blood types and the development of transfusion compatibility in two dog groups (patients undergoing intensive therapy and potential donors) from northwestern Romania, using blood typing and modern monoclonal antibodies tests, based on tube agglutination erythrocytic antigen detection with SHIGETA kits and through card and chromatographic methods with anti-DEA 1.1 kits.

MATERIALS AND METHODS

Animals: The research regarding SHIGETA and DEA 1.1 blood typing and transfusion compatibility was carried out on two heterogeneous dog populations. The tested animals were from Faculty of Veterinary Medicine Cluj-Napoca emergency hospital and clinics (n=70) and also from 5 private veterinary clinics (n=91). One group was composed of 136 dogs of different breeds, the second consisting of 25 dogs. In both groups German Shepherd and Romanian Shepherd were the predominant breeds. Blood samples (1-2 ml) were collected in heparinized tubes for the first group, and on EDTA for the second. The patients in this study were considered as having a guarded or unfavorable prognosis, being diagnosed with hemorrhagic gastroenteritis, blood clotting disorders (produced by anticoagulant rodenticide poisoning), tumoral processes (lymphoma, leukemia), or

hypovolemic shock (due to polytrauma and active bleeding). The transfusion decision was based on clinical assessment, on hematological findings and on blood group compatibility and was performed with whole blood in an average dose of 8.5 ml/kg.

Blood typing and transfusion compatibility: In the first canine group, blood typing was performed using SHIGETA kits and in the second group, with anti-DEA1.1 antibodies kits. Blood type testing and transfusion compatibility assessment were conducted in the FMV Cluj-Napoca Physiology Department laboratory by experienced hematologists.

SHIGETA kit testing: The testing was performed according to the manufacturer's instructions, on heparinized blood, collected from the first group, using the tube agglutination method as main testing procedure, and slide agglutination method as a simplified technique.

Tube agglutination method (TUBE) is based on the evaluation of canine erythrocyte (agglutination) reactions with 4 types of monoclonal antibodies (SHIGETA Animal Pharmaceuticals Inc., Toyama, Japan). According to the compatibility chart, the level of blood compatibility could be established: maximum compatibility, when the two partners have the same blood type, compatibility when two partners have at least 2 common antigens and compatibility accepted only in emergency situations, when some minor transfusional reactions are not excluded (Ognean *et al*., 2005).

Conventional slide agglutination method (SLIDE), consisted of agglutination of PBS erythrocytes suspension with the 4 types of monoclonal antibodies on slide, similar to the method used by Mayank *et al*. (2011) in determining the cat blood types.

Testing with anti-DEA 1.1 antibodies kits: The evaluations using anti-DEA 1.1 antibodies were made through card agglutination and chromatographic tests, according to manufacturer's standard operating procedures.

Card agglutination assay (CARD) is a procedure for the detection of agglutination between DEA 1.1 antigen-bearing erythrocytes and lyophilized specific monoclonal antibodies (RapidVet-H Canine, DMS Laboratories, Neuhausen am Rheinfall, Switzerland). According to the kits instructions, (the) positive reaction in the auto agglutination test invalidates the test and a prolonged reading time (over 2 minutes) can lead to false positives reactions.

The Chromatographic method (CHROM) is based on canine erythrocyte capillary migration on a membrane impregnated with specific monoclonal antibodies (DME VET, Alvedia, Limonest, France). The positive agglutination reaction was expressed through a red band.

Recording and data processing: For each tested blood sample, the results were correlated with breed and clinical status. The obtained results were statistically analyzed and charted using specialized applications such as GraphPad Instat, Microsoft Excel, OriginLab 8.5. We also resorted to extrapolation of the SHIGETA blood types in the DEA system. The data resulted from the donor-recipient compatibility interpretations was the basis for assuring the safety of blood transfusions in the two investigated groups of dogs.

RESULTS

The SHIGETA antigenic system investigation: Blood typing in the first group of dogs showed differences regarding intensity and clarity of the agglutination reaction both in tube and on slide. The maximum agglutination intensity (a single large aggregate) was clearly expressed in tube and on the slide. However, medium reactions (multiple aggregates) were insufficiently clear on the slide, while the low intensity (multiple small aggregates) and very weak (multiple very small aggregates) were non-evident.

The basic characteristic of the SHIGETA antigenic system structure was given by the predominance of the B antigen. As shown in Fig. 1, this antigenic configuration was represented in the highest proportion (90.42%) by the 3 antigen B types, unassociated with antigen A: 1.1B (47.05%), 1(-)B (24.26%) and 1.2B (19.11%). This antigenic structure is also completed by 3 groups of the A antigen, however, these had very low representation: 1.1AB (8.08%), 1.2AB (0.73%) and 1.1A (0.73%).

An overview of blood type incidence in the investigated breeds also highlighted the predominance of the B antigen groups: 1(-)B in German Shepherds (54.16%) and English Bulldogs (50%); and 1.2B in German Pointers (60%). However, the most common was the 1.1B blood type, which has been reported in most of the investigated breeds: Central Asian Shepherd (100%); Rottweiler (81.81%); Romanian Shepherd (73.68%); crossbreds (42.10%). Less homogeneous were the groups of Rottweiler and crossbred dogs, because they included in small amounts types with antigen A (1.1AB 9.09% respectively 10.52%). An increased level of B antigen types was also found in "other breeds" category, in which the frequency of blood types was similar to that of the entire group, the majority being composed of 1.1B (34.28%), 1.2B (31.42%), and 1(-)B (17.14%). According to the obtained data almost half of the tested canine population (45.58%) was composed of German Shepherds, Romanian Shepherds and crossbreds, in which 1.1B and 1(-)B were the predominant blood types.

Investigation of DEA 1.1 antigenic system: The accuracy of the anti-DEA 1.1 antibodies tests performed in the second group of dogs, consisting mainly of patients transfused with whole blood, showed some differences between the two used methods. Thus, the agglutination intensity in CARD testing was low and did not persist after drying, while the positive line in CHROM testing was well marked and sufficiently stable.

Individual and mean data analysis of group two revealed as a predominant characteristic the presence of DEA 1.1 positive dogs, their frequency reaching 75% (Figure 2). This separation of dogs in DEA 1.1 positive and negative revealed a 1 to 4 ratio in the two groups and proved to be of major clinical importance for managing transfusion therapy and for identifying potential donors. Although data from the investigation of these groups does not permit a detailed analysis of the incidence of DEA 1.1 type in breeds, it shows a predominant tendency of negative dogs, known as high-potential donors, in German Shepherds and in mixed breed dogs (Fig. 2).

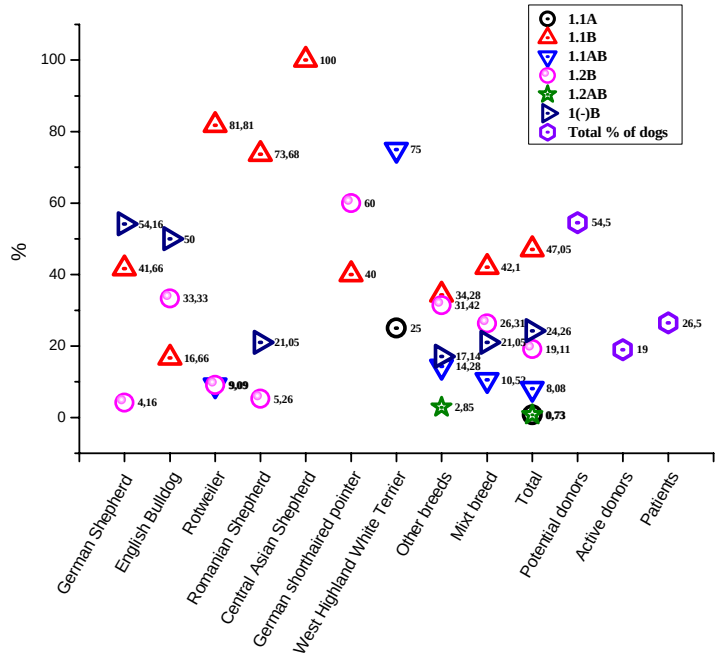

Fig. 1: Frequency of canine SHIGETA blood types in the first group, composed of potential donors (54.5%), active donors (19%) and patients (26.5%).

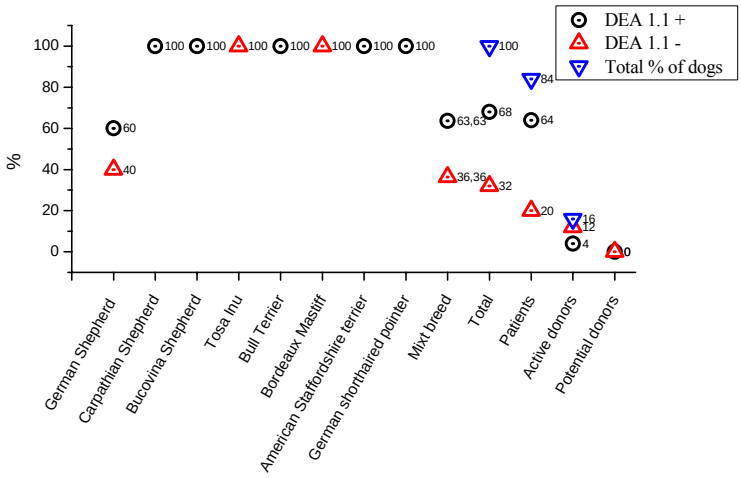

Fig. 2: Frequency of canine DEA1.1 blood types in the second group, composed of active donors (16%) and patients (84%).

Correlation of SHIGETA and DEA blood types: A good assessment of the potential donor sources is required for extrapolation of the SHIGETA types into the DEA system. From this correlative analysis and interpretation chart, it was seen that to the type 1.1B corresponds the DEA 1.1, DEA 4 and DEA 6, and to type 1(-)B corresponds the DEA 4 and 6 types. In this context, the obtained results indicate that German Shepherds are an important source of potential donors. A good potential for compatible blood donors was also attributed to English Bulldogs and Romanian Shepherds due to the increased proportion of 1(-)B positive dogs.

DISCUSSION

The blood typing kits available on the market, especially for the typing of the DEA 1.1 blood type, are considered to be the most effective and reliable therapeutic procedure on this matter (Hale, 1995). The obtained elevated mean values of the TUBE test agglutination reactions, indicated a good detection of erythrocyte antigens by the monoclonal antibodies from the SHIGETA kits, the reduced accuracy of the aggutina- tion reactions of these kits on slide limits the implementa- tion of the slide method even though is less costly.

The tube agglutination method using polyvalent antiserum was the basis for establishing the following DEA blood types distribution in a population of 198 dogs from the Turkish Kangal breed: 1.1 (61.1%); 3 (23.2%); 4 (100%); 5 (55.5%) and 7 (71.1%) (Arikan *et al*., 2009). Previous studies show that the tube agglutination method was initially used to test the DEA 1.1 blood type with polyclonal alloantibodies, obtained by sensibilizing DEA 1.1 negative dogs (Hale, 1995; Kohn *et al*., 2012). The variable agglutination reactions observed in this first

version led to some corrections in the Coombs reagent, based on canine antiglobulin (Giger *et al*., 2005).

In the present study, the found positive reactions were better emphasized with the CHROM than with the CARD method. The immunocromatographic method was superior even when testing samples from severally anemic patients. Other research data regarding the efficiency of the CARD method shows that it is a quick option in identifying the DEA 1.1 blood type, but provides poorly seen reactions in the case of DEA 1.2 positive dogs (Arikan *et al*., 2009; Seth *et al*., 2012). A study conducted on 38 DEA 1.1 positive Dalmatians using the CARD method is also relevant here for the agglutination reactions: 18% low intensity, 29% medium intensity and 53% high intensity (Gračner *et al*., 2011). In some veterinary clinics more complex blood typing tests are being used, as the ones with polyclonal antiserums developed by the University of Michigan State, or the GEL test with anti DEA 1.1 monoclonal antibodies (Giger *et al*., 2005). In the last decade more and more blood typing tests based on monoclonal antibodies for the DEA 1.1 blood type have been standardized, leading to an increase in the production of blood typing kits (Riond *et al*., 2011). The progresses made in the DEA blood typing have failed to provide a larger accessibility to the antiserums. Only 6 of the 13 DEA groups have identifica- tion kits with a wider spread (1.1, 1.2, 3, 4, 5 and 7). Even these are rarely found in veterinary clinics, most practitioners still turn to the DEA 1.1 monovalent card.

The identification of specific alloantibody in a Dalmatian eventually reflects the large diversity of erythrocyte antigens (Blais *et al*., 2007). It is thought that the Dalmatians, who do not have the new antigen, named "Dal", are predisposed to acute or delayed post-transfusion reactions. Some studies focusing on the detection of natural preformed alloantibodies against some DEA types have shown that only anti DEA 7 alloantibodies are formed, with no clinical relevance (Lanevschi and Wardrop, 2001; Kessler *et al*., 2010). This means that gestation will not sensibilize females to the erythrocytic antigen, and that they can be used safely as donors (Blais *et al*., 2007). It is well known that a dog that had more than one blood transfusion will develop alloantibodies against erythrocytic antigens, even if it is compatible by crossmatch with several donors. These alloantibodies are often found (92-99%) in dog populations, mostly as a result of incompatible blood transfusions given to DEA 1.1 negative dogs (Riond *et al*., 2011). This confirms that the best donors are young dogs that are at their first transfusion. In the tested groups, the potential donors were mainly 1(-)B positive DEA1.1 negative German Shepherds.

The data provided by this study regarding the occurrence of the DEA 1.1 group, using CARD and CHROM tests showed a significant proportion of DEA 1.1 positive dogs in the tested group, which is also linked to a small number of potential donors. These tests can be used in a screening process for potential donors, considering DEA 1.1 negative dogs as universal donors (Hale, 1995; Ognean *et al*., 2005).

The distribution of the DEA 1.1 positive blood type within the breeds was under 20% for German Shepherds and Boxers and over 70% for Rottweilers, Great Danes, Saint Bernards and Dalmatians, as German Shepherds and Greyhounds are mostly DEA 1 negative and considered good donors (Van Der Merwe *et al*., 2002). A study performed on healthy dogs from different breeds revealed significant associations between breeds and some of the DEA groups (Esteves *et al*., 2011). For a more precise result, further study of blood compatibility should involve at least three of the major antigenic groups (DEA 1.1, 1.2, 7).

Conclusion: The correlation of SHIGETA and DEA antigenic systems is of major clinic relevance, the 1(-)B dogs belonging mainly to German Shepherd breed, proved to be also DEA 1.1. negative, which ultimately confirms their undoubted value as universal donors.

Based on the obtained data, we assign a good efficacy to the chromatographic method in auto agglutination testing and donor-patient compatibility, including cases of severe anemia.

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